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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

June 26, 1996

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Metam sodium. Registrant Response to Residue Chemistry Reregistration Data Deficiencies. Reregistration Case No. 2390. Chemical I.D. No. 039003. D215298. MRID Nos. 43632600, 43632601, 43632602. CB No. 15996.

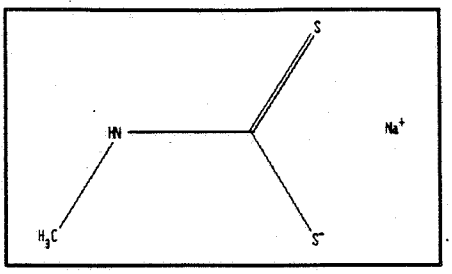
FROM: Stephanie H. Willett, Chemist
Tolerance Petition Section 2
Chemistry Branch I-Registration Support
Health Effects Division (7509C)

THRU: Edward Zager, Acting Branch Chief
Chemistry Branch I- Registration Support
Health Effects Division (7509C)

TO: Tom Myers/Mark Wilhite, PM-51
Accelerated Reregistration Branch
Special Review and Reregistration Division (7508W)

The Metam Sodium Task Force has submitted a turnip metabolism study and the results of an independent laboratory method tryout in response to reregistration data requirements (see most recent memos of F. Fort dated 3/16/95 and 4/14/94). The independent method tryout validates the previously submitted enforcement method (WRC 88-43: MRID No 41142502), as required by PR Notice 88-5. The metabolism study is intended to confirm the results of previously submitted studies which suggested that radioactive residues in plants were due to natural incorporation of ^{14}C from the carbon pool, and not to metam sodium of any of its toxicologically significant metabolites.

Metam sodium is used as a soil sterilant. No tolerances are presently established. A Phase 4 review was completed 5/13/91. The Reregistration Eligibility Decision (RED) for metam sodium is not scheduled for completion in FY 1996. The structure of metam sodium is as follows.



Conclusions

1) The turnip metabolism study is acceptable, and adequately fulfills the data requirements specified in residue chemistry guideline 171-4(a), nature of the residue. CBTS concurs with the registrant(s) conclusions based on this study, which confirms the indications of previously conducted studies. Metam sodium is significantly metabolized in plants. Uptake of soil bound bioavailable residues through roots and translocation to other plant parts is the route of exposure. Since neither metam sodium, MITC nor any related thioureas or methylated ureas were detected in the extractable radioactivity or the post extraction solids, and radioactivity was shown to be distributed over a variety of natural products, the study results indicate complete incorporation of metam sodium into the carbon pool.

2) The MITC method validation report is acceptable. Residue chemistry data requirements as specified in guideline 171-4(c) are considered satisfied.

Recomendations

Based on the information discussed in conclusion 1 above, CBTS concludes that this situation is one in which no tolerances are needed, with respect to the use of metam sodium as a soil sterilant. There are no remaining outstanding residue chemistry data requirements.

Detailed Evaluation

Turnip Metabolism Study

The metabolism study (MRID No. 43632602) was conducted at American Agricultural Services, Inc, in Lucama, NC from October 13, 1993

through April 4, 1994. An outdoor flat of sandy loam soil (6 ft^2 plot) was treated with ^{14}C metam sodium (>90% purity; radiolabeled at carbon disulfide bond) by drenching the soil at a rate of 318 lbs ai/acre (3.31 g ai/ft^2,, for a total of 19.8 g ^{14}C metam sodium), which is maximum label rate. The dose could not be exaggerated without phytotoxic effect to the crop, and because of inherent environmental radiation hazards due to the volatile dissipation of the test substance. Turnips were planted at 7, 10, 14 and 21 days after treatment (DAT). The 7-DAT crop did not survive. The 10-DAT crop showed some symptoms of phytotoxicity, but recovered and grew to maturity and were analyzed along with the 21-DAT crop, which grew normally. The 21-DAT crop represented the label recommended conditions.[1] Samples of mature tops and roots from the 10-DAT plot were harvested at 150 days after planting (160 DAT), and from the 21-DAT plot at 152 days after planting (173 DAT). Tops and roots were separated and stored frozen in plastic bags immediately after harvest (<-20°C) until shipment. Plants grown in control plots were handled identically.

Treated plants arrived at Battelle Laboratory in Columbus, Ohio one day (10-DAT samples), or fourteen days (21-DAT samples) after harvest and were kept frozen until analyzed. All residue analyses were conducted according to current GLP Practices, and measures were taken throughout the study to assure that study results would be considered valid (e.g. use of controls, instrument calibration, and cross contamination preventing procedures, etc.). Total radioactive residue levels (TRR) were determined by combustion/LSC. All samples were homogenized and combusted for determination of total radioactivity levels within 45 days of receipt at the lab. Turnips planted 10-DAT and 21-DAT demonstrated significant uptake of radioactive residues in both roots (2.36 ppm 10-DAT; 3.18 ppm 21-DAT) and tops (2.83 ppm 10-DAT; 4.81 ppm 21-DAT). The 21-DAT samples were not analyzed beyond TRR determination since the radioactivity levels were comparable to those in the 10-DAT samples.

The 10-DAT turnip roots and tops were homogenized separately, followed by exhaustive extractions with acetonitrile:water (1:1), then hexane. The ACN:water extracted 81.1% of the TRR in tops, and 65.5% of the TRR in roots. Since less than 1% of the TRR (<0.024 ppm roots and <0.028 ppm tops) was extracted into hexane, no additional analyses were performed on that phase. Extractions were performed with a minimum of two replicates to confirm reproducibility and provide adequate material for analysis.

The ACN:water extract was frozen at -20°C, thus promoting separation into an acetonitrile phase, and a (solid) aqueous phase. Upon separation and analysis of the two phases by LSC, the majority of the extracted radioactivity was determined to have remained in

[1]Since the 14-DAT crop was not needed, they were removed as seedlings and were not analyzed.

the aqueous phase (>89%), with a much smaller amount (11%) remaining in the acetonitrile phase.

Upon qualitative TLC analysis of the aqueous phase extracts of tops and roots in a solvent system of dichloromethane:methanol: water:acetic acid (65:25:4:4, v:v), hereafter referred to as SS1, a total of eleven different "metabolites" were determined to be present in both tops and roots with three (M2, M3, M4) being unique only to tops, and one (M6) being unique only to roots. Only three (M9, M10, M11) of the eleven were present at levels sufficient enough to allow further characterization/identification (i.e. >10% TRR, 0.05 ppm). It should be noted that TLC analysis of the aqueous phase extracts in acetonitrile (SS2), a less polar mobile phase, was unable to separate the aqueous phase extracts. Standards of parent metam sodium and related compounds were well resolved in both solvent systems. No extractable radioactive residues co-chromatographed with parent metam sodium, or related substituted thioureas (MITC, MTU, DMTU, DMU; see attachment 1 for structures). See table 1 for a summary of the results of the characterization analyses.

TABLE 1: SUMMARY OF CHARACTERIZATION OF RADIOACTIVE RESIDUES IN ^{14}C Metam sodium 10 DAT TURNIPS

TLC FRACTION	NATURE OF TLC COMPONENT[1]	%TRR IN ACN PHASE		% TRR IN AQUEOUS PHASE		TOTAL % RADIOACTIVITY		PPM[2]	
		TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS
1	Rf 0.95	2.1	1.7	3.6	---	5.7	1.7	0.161	0.040
2	Rf 0.87	0.6	---	---	---	0.6	---	0.017	---
3	Rf 0.79	0.6	---	---	---	0.6	---	0.017	---
4	Rf 0.68	0.4	---	---	---	0.4	---	0.011	---
5	Rf 0.47-0.53	0.8	1.0	4.1	---	4.9	1.0	0.139	0.024
6	Rf 0.49	---	0.5	---	---	---	0.5	---	0.012
7	Rf 0.41-0.42	0.4	0.3	3.3	4.2	3.7	4.5	0.105	0.106
8	Rf 0.34-0.35	---	0.2	6.7	5.5	6.7	5.7	0.190	0.135
9	Rf 0.25-0.27	0.5	0.2	19.9	14.9	20.4	15.1	0.577	0.356
10	Rf 0.019-0.20	0.9	0.3	26.9	10.7	27.8	11.0	0.787	0.260
11	Rf <0.1	---	---	13.6	25.4	13.6	25.4	0.385	0.599
	hexane partition	---	---	---	---	0.1	1.0	0.004	0.023
Extractable Subtotal		6.3	4.2	78.1	60.7	84.5	65.9	2.39	1.556
Nonextractable Subtotal		na	na	na	na	21.1	38.1	0.597	0.091
TOTAL		na	na	na	na	105.6	104.0	2.99	2.454

1 TLC SS1 Rf VALUE USING THIN LAYER CHROMATOGRAPHY SYSTEM FOR POLAR METABOLITES.
2 PPM IN ^{14}C METAM SODIUM EQUIVALENTS = TOTAL %TRR X 2.83 PPM (TOPS) OR 2.36 PPM (ROOTS)

5

Additional analyses of "metabolite" regions 9, 10, and 11 included extraction/hydrolysis with mild (1N) and strong (6N) acids/bases, and various enzymes (cellulase, pectin, starch) to analyze for conjugates. HPLC was used in an attempt to further identify the subsequently released radioactivity using two different sets of conditions to analyze parent metam sodium and carbohydrates. Metam sodium related compounds (see attachment 1) were shown to be detectable by HPLC/MS.

Metabolite 9 (15.1% TRR, 0.356 ppm in root; 20.4% TRR, 0.577 ppm in tops), isolated in bulk by TLC SS1, was a tightly-concentrated well-resolved band. Upon additional analysis, metabolite 9 degraded under strong basic conditions, but was not affected by mild acid or base hydrolyses, strong acid hydrolysis, or carbohydrate and pectin specific hydrolyses. Organic acids (e.g. fumaric) and amino acids (e.g. l-alanine) had TLC retention times in the region of metabolite 9. An attempt to identify the components was made using liquid chromatography/mass spectroscopy (HPLC/MS) analysis of isolated metabolite 9 from root was not able to discern a parent ion above competing ion traces from matrix (spectra included in report). Root and top analyses were essentially the same.

Metabolite region 10 (11.0% TRR, 0.260 ppm in root; 27.8% TRR, 0.787 ppm in tops), isolated by TLC SS1, was a relatively wide band which comprised a mixture of components, mainly sugars. ^{14}C-Glucose co chromatographed in part with metabolite region 10 using both TLC SS1 for roots and tops, and carbohydrate HPLC for root. Metabolite region 10 falls in a zone for mono and disaccharides framed by ^{14}C-glucose and ^{14}C-maltose. Harsh acid hydrolysis and derivation of region 10 components to ^{14}C-osazones gave final confirmation of metam sodium incorporation into reducing sugars.

Metabolite region 11 (the mixture of polar material retained at the origin using TLC SS1, 25.4% TRR, 0.599 ppm in root; 13.6% TRR, 0.385 ppm in tops) cochromatographed with ^{14}C-starch. Both Metabolite region 11 and ^{14}C-starch eluted in the void volume on the carbohydrate HPLC column. Metabolite region 11 was more abundant in the aqueous roots extract than the aqueous tops, which further indicated that the components of this phase were carbohydrates. Additionally, when metabolite region 11 was subjected to acid and base hydrolysis, there was an increase in the activity in region 10 (i.e. glucose was a major product). Metabolite region 11 was enzymatically hydrolyzed with cellulase, pectinase and alpha-amylase indicating it is a mixture of oligomeric carbohydrates. Harsh acid hydrolysis and derivatization of metabolite region 11 to ^{14}C-osazones further confirmed that metabolite region 11 contained complex carbohydrates.

Upon sequential hydrolysis of post extraction solids (i.e. bound residues; 38.1% TRR, 0.901 ppm in roots; 23.2% TRR, 0.657 ppm in tops) with enzymes selective for cellulose, starch, protein, and

pectin, results showed a near even distribution of ^{14}C-residues were released across all classes of natural products represented. A final chemical extraction for lignin from the post-enzyme extracted PES also indicated incorporation of ^{14}C-residues. Since ^{14}C-glucose was previously identified in the extractable residues, incorporation into the carbon pool (shown through enzymatic treatments) via glucose metabolism is highly probable.

Treated tops and roots were extracted twice during the course of the study. The extractability of the radioactive residues was shown to be comparable by LSC, and qualitative TLC profiles of the root and top extracts on both occasions indicated that no significant decomposition had occurred during the time lapse between extractions, which was a period of approximately 14 weeks.

Since the ACN phases of the initial root and top extractions contained greater than 10% of the TRR (see table 1), they were also subjected to additional analyses. When the ACN extracts of tops and roots were analyzed along with parent related standards (see attachment 1) in SS1, all ^{14}C metabolites eluted from the origin, but all were present at levels $\leq$0.05 ppm, except one in the tops extract, which was present at a level of 0.059 ppm. When analyzed by SS2 (acetonitrile), no single component (out of approximately 10) accounted for more than 0.031 ppm. Therefore no further analysis was attempted.

Details of calculation procedures (i.e. dpm to %TRR and ppm conversions) were included in the report. The entire extraction/fractionation scheme employed in the study is summarized in attachment 2 of this memo.

Based on the study results, the registrant(s) have concluded that metam sodium is significantly metabolized in turnip roots and tops. Although radioactive residues could have been taken up by respiration through the leaves of $^{14}CO_2$ dissipating from the soil, or from soil bound residues through the roots, it is speculated that volatile dissipation is expected to have been completed before planting. Thus uptake of soil bound bioavailable residues through the roots and translocation to the tops is suspected to be the route of uptake.[2] Since neither metam sodium, MITC nor any related thioureas or methylated ureas were detected in the extractable radioactivity or the post extraction solids, and radioactivity was shown to be distributed over a variety of natural products, the study results indicate complete incorporation of metam sodium into the carbon pool.

CBTS concludes that the metabolism study is acceptable, and adequately fulfills the data requirements specified in residue

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[2] ^{14}C levels (metam-sodium equivalents) ranged from 27.7 ppm ($\pm$0.6) at 0 days after treatment to 16.6 ppm ($\pm$2.1) at 160 days after treatment. Details of soil analyses are included in the report.

chemistry guideline 171-4(a), nature of the residue. CBTS concurs with the registrant(s) conclusions based on this study, which confirms the indications of previously conducted studies (see also 5/13/91 phase 4 reviews). Collectively, these studies indicate that metam sodium is significantly metabolized in plants, and taken up from soil bound bioavailable residues through roots and translocates to other parts in the plants. Since no discrete residues have been identified in any of the studies (i.e. metam sodium, MITC nor any related thioureas or methylated ureas), and radioactivity was shown to be distributed over a variety of natural products, the study results indicate complete incorporation of metam sodium into the carbon pool.

Enforcement Analytical Methodology

The Metam Sodium Task Force has submitted the results of an independent laboratory validation of method WRC 88-43 (MRID 41142502, pp. 172-187), as required by PR Notice 88-5. Method WRC 88-43 determines MITC (methyl isothiocyanate), a potential metabolite of metam sodium with toxicity concerns, in a variety of raw agricultural commodities (see structure in attachment 1). The method validation was conducted at Zeneca Agrochemicals Laboratories, Jealott's Hill Research Station, Berkshire, UK. The method was developed and previously validated at Zeneca Ag Products, Western Research Station, USA.. No communication took place between the method authors and the personnel conducting the independent validation.

Untreated samples of cabbage were fortified with MITC at levels of 0.028 and 0.1 ppm using working solutions prepared with standard (97%) MITC, along with dilute nitric acid and ethyl acetate. Cabbage was the commodity of choice since it was considered difficult to analyze because of the large number of coextractives. After blending and centrifugation, an aliquot of the ethyl acetate layer is taken and analyzed using gas chromatography equipped with a nitrogen specific thermionic detector. The detector response was shown to have a linear response by injecting standards over an appropriate range (0.01 - 0.1 ug/ml MITC). Blank cabbage samples were extracted and analyzed concurrently with spiked samples.

During a second method tryout, recoveries of MITC at the 0.028 ppm level were 94% and 100%, and 89% and 96% at the 0.1 ppm level.[3] The analysis of a single sample set was completed by one person in five hours. The method validation was therefore considered successful. Chromatograms were included in the report.

[3]The initial method tryout produced recoveries of 102 to 128%, which was out of the acceptable range of 70 to 120%.

CBTS concludes that the MITC method validation report is acceptable. Residue chemistry data requirements as specified in guideline 171-4(c) are considered satisfied.

Attachments: 1) Structures for Metam sodium and related compounds (from page 52 of report)

2) Summary of Extraction and Fractionation Scheme Used in Turnip Metabolism Study (from page 67 of report)

cc: RF, SF, P. Deschamp (RCAB/HED), E. Haeberer, S. Willett, Metam Sodium List B File

7509C::CM2:RM804C:305-6380:SHWillett:shw-6/20/96
RDI: R. Perfetti, 6/24/96; E. Zager, 6/26/96

TABLE I: REFERENCE STANDARDS

COMPOUND NAME [CAS NUMBER]	ABBREVIATION	FORMULA	MOLECULAR WEIGHT	SOURCE (Lot #)	PURITY
1,1-dimethyl urea [598-94-7]	1,1-DMU	$(CH_3)_2NC(=O)NH_2$	88.11	Aldrich (03123CF)	99%
1,3-dimethyl urea [96-31-1]	1,3-DMU	$CH_3NHC(=O)NHCH_3$	88.11	Aldrich, (PF09119KY)	99%
1,3-dimethyl-2-thiourea [534-13-4]	1,3-DMTU	$CH_3NHC(=S)NHCH_3$	104.18	Fluka, (315151/1-593)	99%
1,1,3-trimethyl urea [632-14-4]	1,1,3-TMU	$(CH_3)_2NC(=O)NHCH_3$	102	Alfa AESAR (I07D17)	100%
1-methyl-2-thiourea [598-52-7]	1-M-2-TU	$CH_3NHC(=S)NH_2$	90.15	Aldrich, (AF02103OX)	97%
methyl isothiocyanate [556-61-6]	MITC	$CH_3N=C=S$	73.12	Aldrich, (3126JX)	97%
sodium N-methyl-dithiocarbamate [137-42-8]	metam-sodium	$C_2H_4NNaS_2$ $(CH_3)NHC(=S)S^-Na^+$	129.2	Buckman Labs (839-170B)	>95%
sodium N-methyl-[thiocarbonyl-^{14}C] dithiocarbamate [137-42-8]	^{14}C-metam-sodium	$(CH_3)NH^{14}C(=S)S^-Na^+$	129.2	Wizard Labs (930421)	>90%

Attachment 1

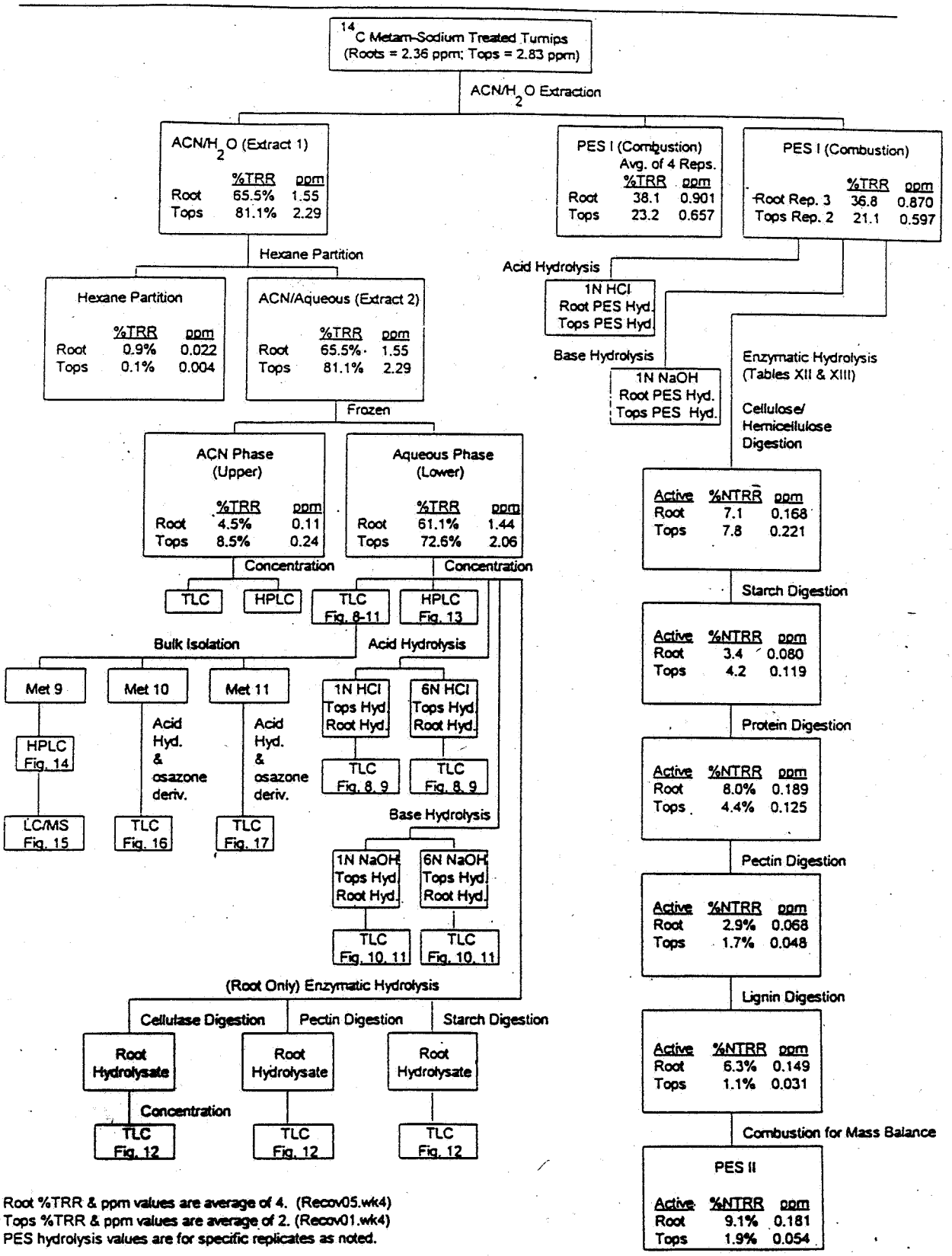

Figure 3: Comprehensive Extraction and Fractionation Scheme Employed for Mature 10-DAT Turnip Roots and Tops Grown in ^{14}C-Metam Sodium Treated Soil.